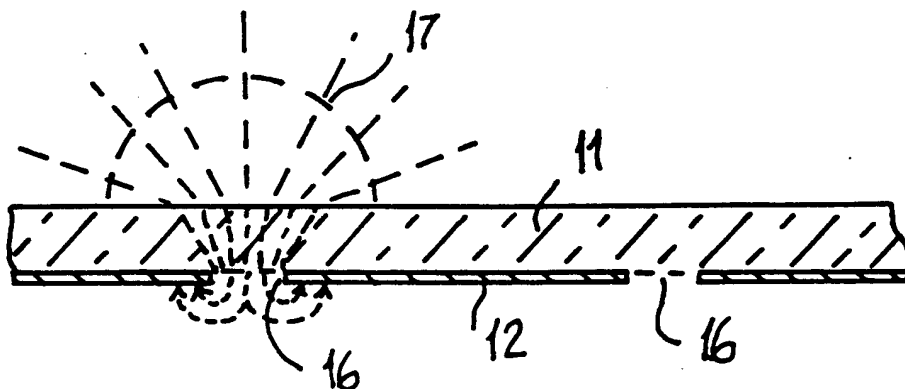


INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : G01N 27/49, C12M 1/40		A1	(11) International Publication Number: WO 94/08236
			(43) International Publication Date: 14 April 1994 (14.04.94)
<p>(21) International Application Number: PCT/GB93/02076</p> <p>(22) International Filing Date: 6 October 1993 (06.10.93)</p> <p>(30) Priority data: 9221099.6 7 October 1992 (07.10.92) GB</p> <p>(71) Applicant (for all designated States except US): ECOSSENSORS LIMITED [GB/GB]; Unit 15, Hanborough Park, Long Hanborough, Oxon OX8 8LH (GB).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (for US only) : TIETJE-GIRAULT, Jordis [DE/CH]; En Vernay, CH-1088 Ropraz (CH). SEDDON, Brian, Jeffrey [GB/CH]; 37, chemin de Fonadel, Prilly, CH-1008 Lausanne (CH). McALEER, Jerome, Francis [GB/GB]; 52 Nobles Close, Grove, Wantage, Oxon OX12 0NR (GB).</p>			<p>(74) Agent: NEWBY, John, Ross; J.Y. & G.W. Johnson, Funnival House, 14-18 High Holborn, London WC1V 6DE (GB).</p> <p>(81) Designated States: CA, GB, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>

(54) Title: GAS PERMEABLE MEMBRANES FOR AMPEROMETRIC GAS ELECTRODES AND USES THEREOF



(57) Abstract

A method of manufacturing a gas-permeable membrane (11) for an amperometric gas electrode comprises demetallising areas (16) of a metallised film (12) to obtain a regular array of gas-permeable micropores. Devices incorporating gas-permeable membranes used as electrodes are disclosed inter alia for analyte determinations and oxygen (e.g. BOD) measurements.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NE	Niger
BE	Belgium	GN	Guinea	NL	Netherlands
BF	Burkina Faso	GR	Greece	NO	Norway
BG	Bulgaria	HU	Hungary	NZ	New Zealand
BJ	Benin	IE	Ireland	PL	Poland
BR	Brazil	IT	Italy	PT	Portugal
BY	Belarus	JP	Japan	RO	Romania
CA	Canada	KP	Democratic People's Republic of Korea	RU	Russian Federation
CF	Central African Republic	KR	Republic of Korea	SD	Sudan
CG	Congo	KZ	Kazakhstan	SE	Sweden
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovak Republic
CM	Cameroon	LU	Luxembourg	SN	Senegal
CN	China	LV	Latvia	TD	Chad
CS	Czechoslovakia	MC	Monaco	TG	Togo
CZ	Czech Republic	MG	Madagascar	UA	Ukraine
DE	Germany	ML	Mali	US	United States of America
DK	Denmark	MN	Mongolia	UZ	Uzbekistan
ES	Spain			VN	Viet Nam
FI	Finland				

- 1 -

GAS PERMEABLE MEMBRANES FOR AMPEROMETRIC GAS ELECTRODES AND USES THEREOF

BACKGROUND OF THE INVENTION

1. Field of the Invention

5 The electrochemical amperometric detection of redox gases such as oxygen is a well established technique, and the electrode used in such a technique is often referred to as a "Clark Electrode". In the case of an oxygen Clark Electrode, this detection is based on oxygen transport
10 through a gas-permeable membrane to an enclosed electrolyte solution, and the subsequent reduction of oxygen dissolved in this solution, usually on a platinum or gold sensing electrode. The potential of this sensing electrode is held at negative potential compared to the potential of the
15 electrolyte solution by use of a reference electrode, classically a silver/silver chloride electrode. A schematic diagram illustrating this conventional approach is shown in Figure 1 of the accompanying drawings.

The response time of classical detection devices, where
20 the sensing electrode, on which the electrochemical reaction takes place, is separated from the gas-permeable membrane by a thin (e.g. a submillimeter thick) layer of electrolyte solution, is more than 100 seconds. The response time is limited by the linear diffusion rate of the redox gas
25 through the gas-permeable membrane and into the electrolyte solution as depicted in Figure 1.

The present invention is based on the use of gas-permeable polymer films (for example films a few microns thick of e.g. polypropylene or polyester) metallised on one
30 side (e.g. with gold or platinum). Such metallised films are commercially available and are currently used in the food packaging industry.

At the heart of the present invention is the use of a novel type of composite gas-permeable membrane which has

- 2 -

been manufactured by demetallising (e.g. by using UV excimer laser photoablation) areas of a metallised polymer film, to obtain a regular array of gas-permeable micropores each having a diameter or width of a few microns. The micropores
5 can be in the form of microdiscs and/or microbands, since the shape of each area is of secondary importance.

2. Description of the Related Art

Our prior International application published as WO 9108474 discloses the use of photoablation for the creation
10 of apertures in electrically insulating material when creating microelectrodes and EP-A-0494382 discloses the creation of an electrochemical cell in which photoablation is used to drill holes in an insulating substrate of the cell and to expose metallised areas on the substrate. EP-A-
15 0494382 does disclose a gas-permeable membrane but not one subjected to subsequent thinning (e.g. by photoablation).

SUMMARY OF THE INVENTION

According to one aspect of the invention there is provided a method of manufacturing a gas-permeable membrane
20 for an amperometric gas electrode from a polymer film metallised on one surface thereof which method comprises demetallising areas of the metallised film to obtain a regular array of gas-permeable micropores having a diameter or width of a few microns.

25 The polymer film can be inherently gas-permeable when demetallised, but if made of non-permeable material can be made gas-permeable over the localised areas where demetallisation is effected.

Conveniently, the regular array of micropores is
30 obtained by excimer laser photoablation, preferably using a UV excimer laser. The metallised film is desirably of gold or platinum and the polymer film preferably is polypropylene or polyester.

- 3 -

Suitably each micropore comprises a porous plug replacing film material removed in the demetallising process.

An amperometric gas electrode made by the method of the invention represents a further aspect of this invention as do disposable devices using such electrodes and incorporating enzymes or microbes for controlled biological testing methods.

BRIEF DESCRIPTION OF THE DRAWINGS

10 The invention will now be further described, by way of example, with reference to the accompanying drawings, in which:

Figure 1 is a schematic view of a classical amperometric oxygen electrode,

15 Figure 2 is a schematic view of a first embodiment of electrode in accordance with this invention,

Figure 3 is a view of the diffusion field applicable to an electrode as shown in Figure 2,

Figures 4 and 5 are views corresponding to Figures 2 and 3 applicable to a second embodiment of electrode in accordance with the invention,

Figure 6 is a schematic sectional side view of a composite gas-liquid-liquid electrode in accordance with the invention,

25 Figure 7 is a schematic cross-sectional view of a cell for determining BOD or sample toxicity according to the invention,

Figure 8 shows one method of making an electrode

- 4 -

membrane for use in the electrodes of Figures 2 and 4,

Figure 9 schematically illustrates one form of electrode mounted on a support, and

Figure 10 is an enlarged sectional view of a modified arrangement of electrode and support therefor.

DESCRIPTION OF PREFERRED EMBODIMENTS

As already discussed Figure 1 illustrates a prior art arrangement in which the oxygen gas permeates through a permeable membrane 11 into an electrolyte solution 14 where
10 chemical reaction occurs in the presence of a sensing electrode 12 and reference electrodes 15.

An oxygen electrode device according to the present invention which includes a novel gas-permeable membrane is illustrated in Figure 2 and comprises a gas-permeable
15 polymer film 11 having a metallic electrode layer 12 provided with demetallised microareas 16, a thin layer of an electrolyte solution 14 and a reference electrode 15 which can be a silver/silver chloride electrode.

Because oxygen cannot permeate through the metallic
20 layer 12, oxygen flow between the analyte and the internal electrolyte solution 14, can only occur through the demetallised microareas 16. The oxygen flowing through these areas can then be classically electrochemically reduced. The collection efficiency of the metallic
25 electrode layer 12, e.g. of gold or platinum, for the reduction of the oncoming oxygen is very high due to the micronic dimension of the demetallised areas. Furthermore, the electrode layer 12 cannot be closer to the membrane 11 than in the arrangement illustrated, and therefore the need
30 for diffusion of oxygen through the electrolyte solution 14 is obviated. Both of these innovative features combine to provide improvements in the oxygen electrode performance.

- 5 -

The flux of oxygen through the gas-permeable membrane 11 however is not homogeneous as in a classical oxygen electrode (employing flux by linear diffusion as shown by dashed line arrows in Figure 1) but converges to each 5 demetallised microarea 16 as shown by the dashed-line arrows in Figure 3. In the case of the microareas 16 being microdiscs, this results in a pseudo hemispherical diffusion regime, where each demetallised microdisc 16 acts as a sink for the oxygen present in a hemispherical diffusion shell 17 10 above it, an arrangement expected to lead to a steady state flux of oxygen with reduced dependence on the flow of the analyte above the membrane 11. The oxygen flux density through the microdisc 16 is much higher than that provided by simple linear diffusion processes; thus providing higher 15 local oxygen concentration near the reducing electrode 12.

A further advantage of a manufacturing method according to this invention is that the thickness of the gas-permeable membrane 11 can be reduced by the demetallising process (e.g. if more laser pulses than those necessary for a 20 demetallisation photoablation process are used). This allows further tailoring of the permeation properties of the membrane 11 and therefore leads to a reduction of the response time of the electrode device.

A modification of the invention is to use the same 25 demetallising agent (e.g. a UV excimer laser photoablation system), not only to demetallise over the required areal pattern but also to drill at least partly through the thin polymer layer. When drilling fully through the polymer film (as shown in Figure 4) it is not necessary to use a gas- 30 permeable polymer film since the microhole arrangement so created can then be filled by a plug 18 of a phase which is highly oxygen permeable, e.g. a plug of silicon material. This hole filling or plugging procedure can be carried out, for example, by a screen printing method or a casting 35 method. In this modified approach, the array of filled microholes represent an array of oxygen micropores and

- 6 -

because the dimensions of the filled microholes are on the micron scale, the diffusion of oxygen to these microdiscs is controlled by a regime of part-spherical diffusion similar to that observed for the diffusion of reactants to a 5 microdisc electrode array of similar dimensions created in a still coherent gas-permeable film as shown in Figure 2.

The difference in the gas diffusion fields between the classical case, i.e. linear diffusion, and the part-spherical gas diffusion field which arises with the use of 10 an electrode according to this invention is thought to be the main source of the advantages achieved by the present patent application. Indeed, linear gas diffusion fields cannot yield steady state regimes and any technique based on this type of mass transfer requires a calibration procedure. 15 In contrast, the use of a part-spherical diffusion field is by nature much more efficient as it gathers redox gas from a wider volume, and consequently yields a steady state gas diffusion field. In the case of the amperometric determination of oxygen, this means the generation of a 20 constant reduction current which is a function of the geometry of the device, the diffusion coefficient of oxygen (which is a function of temperature) and the concentration it is wished to measure. If the geometry of the electrode device and the temperature are known, then the concentration 25 can be directly and accurately calculated without resorting to time-consuming calibration procedures.

This generic proposed method for the amperometric detection of oxygen is particularly well suited for the design of biosensors where an enzyme (E), say glucose 30 oxidase, competes with the device for the oxygen dissolved in the analyte. Many biosensors based on the classical oxygen electrode have been proposed. The response of the devices hitherto proposed is limited by the physical distance between the enzymes, the membrane and the 35 electrode. By the nature of the proposed novel design, the enzymes can be immobilised on the thin polymer film. In this

- 7 -

way, the distance between the enzyme-membrane and the membrane-electrode is reduced which in turn results in a reduced response time. Although enzymes can also be immobilised on the gas-permeable membrane of a classical "Clark Electrode", they tend to hinder the linear flux of oxygen. In the case of an electrode according to this invention however, the "active" surface, i.e. the surface area directly above the demetallised microdiscs represents less than 1% of the total surface area, yet the partial spherical diffusion fields can be arranged to cover the majority of the sensor surface by an appropriate choice of the density of the array of micropores. It is possible to limit the enzyme immobilisation in areas not overlaying the demetallised microareas by using the modified approach of creating filled micropores. Indeed, the difference in materials used for the membrane 11 and the micropore filling material phase 18 allows the enzyme immobilisation to occur only on the film but not on the micropore filling phase.

Coupling with oxidases

Numerous analytes can be determined via oxygen depletion in the presence of the appropriate enzyme. For example, cholesterol reacts with oxygen in the presence of cholesterol oxidase to form cholestenone. The rate of this reaction depends on the concentrations of enzyme and substrate and on the partial pressure of oxygen in the sample. Thus a simple device for determining cholesterol would comprise an electrode or array of electrodes for determining the background oxygen tension and an enzyme modified array for determining oxygen tension in the presence of the enzyme reaction (i.e. in the manner shown in Figure 3). The enzyme could be immobilised onto local areas of the surface of the gas membrane or alternatively the sensor could comprise two compartments, one containing the enzyme and one without. The cholesterol concentration would conveniently be calculated by comparing the oxygen responses on the two parts of the sensor. The same principle could be used to determine many other analytes. for example glucose

- 8 -

(using glucose oxidase), phenols (using polyphenol oxidase), xanthine (using xanthine oxidase) and so on.

Similarly, other analytes can be determined by the amperometric detection of enzymically produced ammonia. Examples are urea (using urease) and creatinine using creatinine imminase. The amperometric determination of ammonia can be effected either by inclusion of a redox marker species such as bromocresol green in an electrolyte solution 14 shown in Figure 6 or by transfer of the ammonium ion across a supported liquid-liquid interface. In Figure 6, ammonia diffuses across a gas-permeable membrane 11 and dissolves as ammonium ions in the electrolyte solutions in the aqueous phase 14. Each ammonium ion is then driven across the interface into a supported non-aqueous phase 40 by the application of an appropriate potential difference between the electrodes 12 and 15. The ammonia concentration can then be calculated from the ion transfer current.

Biological Oxygen Demand

In specific embodiments of a device according to the invention an oxygen electrode can be used in conjunction with microbes to determine biological oxygen demand (BOD) or toxicity. Thus in Figure 7 the respiration rate of microbes (e.g. freeze-dried microbes) held in a layer 41 in close proximity with a gas-permeable membrane 11 is determined by the BOD of the sample. A high BOD will result in a rapidly changing oxygen tension whereas a low BOD will result in a fairly constant oxygen measurement. One of the main advantages the micro-electrode configuration brings to this measurement is that the micro-electrode itself consumes very little oxygen and so does not perturb the measurement. A total toxicity test kit would include a layer 41 loaded with microbes and nutrient next to the gas-permeable membrane. Since both of these devices measure oxygen depletion in the sample, a sample chamber 42 is included to minimise oxygen ingress from the atmosphere.

- 9 -

An oxygen electrode or biosensor in accordance with this invention can be manufactured by a continuous reel to reel process, as shown in Figure 8 where a web 30 of metallised film passes from a supply spool 31 to a wind-up 5 spool 32. In its passage between spools a pattern of apertures set by a mask 33 is formed in the web 30 by photoablation at a station 34 using ultra violet laser light 35 from a source not shown. The ablation can be effected "on the fly" or in a dwell period following each advance of 10 the web by a pre-set amount. Downstream of the photoablation station 34 is a hole-filling station 36 where the holes formed in the web at station 34 are filled with an appropriate plug of gas-permeable material. A squeegee 37 schematically illustrates this stage.

15 In addition to the two types of system discussed in Figures 6 and 7 further systems are possible using a filled web as created by the process shown in Figure 8.

Firstly, a stick electrode approach can be employed where the film is secured to (e.g. clipped on) a body 20 containing the reference electrode, and the electrolyte solution; very much like a prior art commercially available oxygen electrode. The main difference being that the platinum or gold electrode on which the electrochemical reduction takes place is attached to the gas-permeable 25 membrane, and that therefore electrical contact should be made to the latter (see Figure 9). In Figure 9 the reference electrode is shown at 19, the containing body at 20, and a metal contact to the metallised film of membrane 21 is shown at 22. Neither the film nor the clip is shown 30 in Figure 9.

Figure 10 shows an arrangement in which a web 11 of polymer film having a working electrode 12 on its lower face and containing plugged micropores 16 is supported above a support 19 carrying a reference electrode 15. Spacers 20 of 35 dielectric material hold electrodes 12 and 15 apart to

- 10 -

contain a layer of electrolyte 14 therebetween. Both of the electrodes 12 and 15 and the spacers 20 can be created by screen printing and the electrolyte solution can also be printed as part of the process (i.e. it can be in the form of an aqueous gel or hydrogel).

- 11 -

CLAIMS

1. A method of manufacturing a gas-permeable membrane for an amperometric gas electrode from a polymer film metallised on one surface thereof which method comprises demetallising areas of the metallised film to obtain a regular array of gas-permeable micropores having a diameter or width of a few microns.
2. A method according to claim 1, in which the polymer film is inherently gas-permeable when demetallised.
3. A method according to claim 1, in which the polymer film is of non gas-permeable material but is rendered gas-permeable over the localised areas of said regular array of micropores.
4. A method according to any one preceding claim, in which the regular array of micropores is obtained by excimer laser photoablation.
5. A method according to claim 4, in which the photoablation involves the use of a UV excimer laser.
6. A method according to any one preceding claim, in which the metallised layer on the polymer film is of gold or platinum.
7. A method according to any one preceding claim, in which the polymer film is polypropylene or polyester.
8. An amperometric gas electrode made by the method of any one preceding claim.
9. A redox gas detection device incorporating a gas electrode as claimed in claim 8.
10. An amperometric gas electrode made by the method

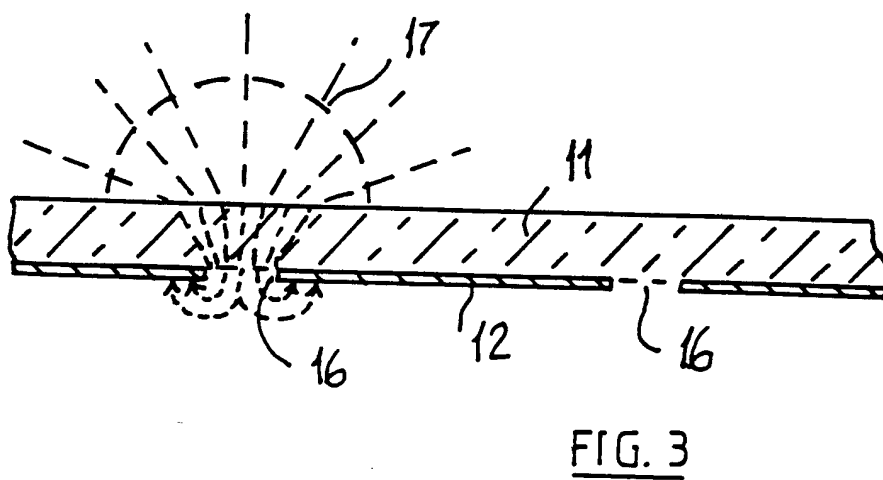
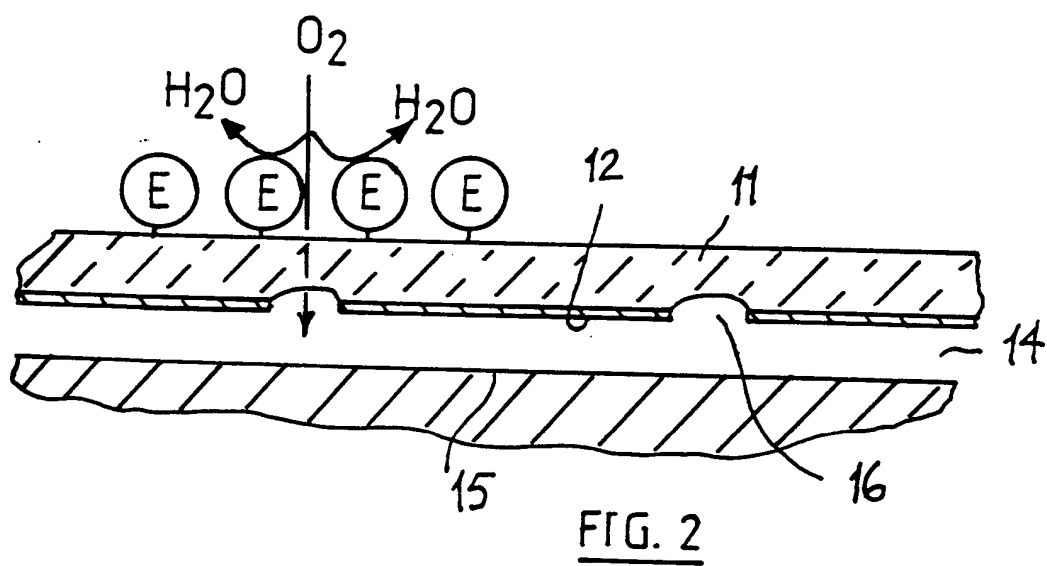
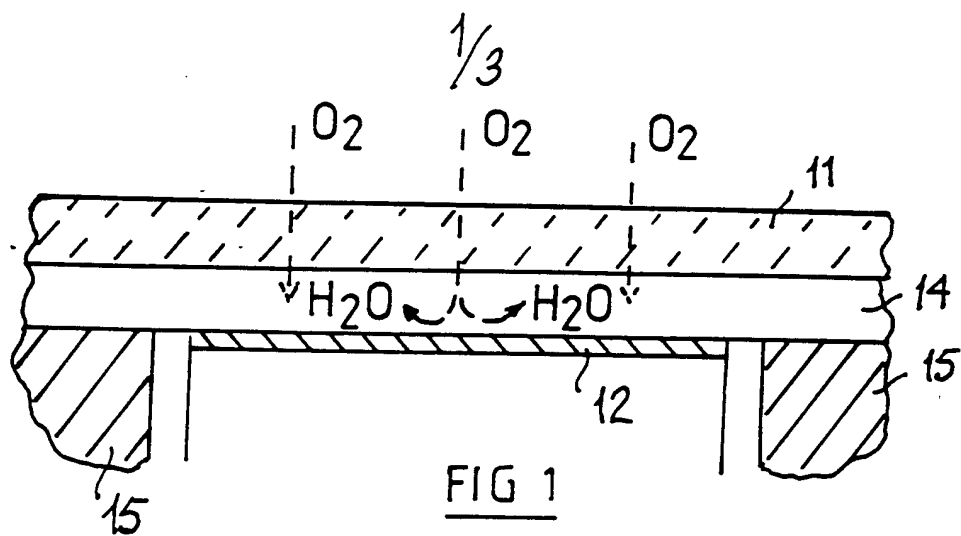
- 12 -

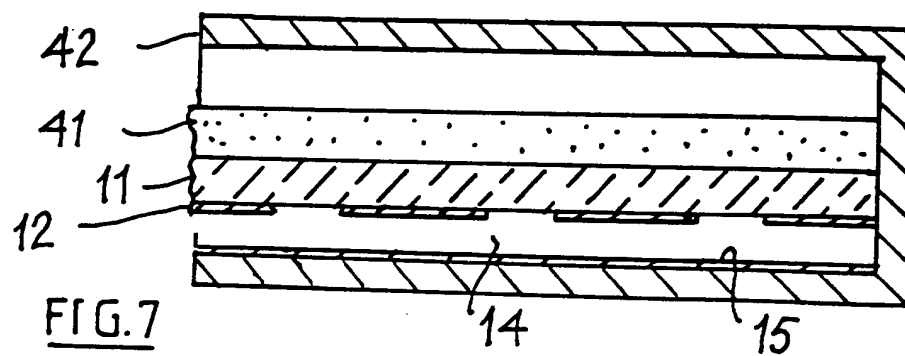
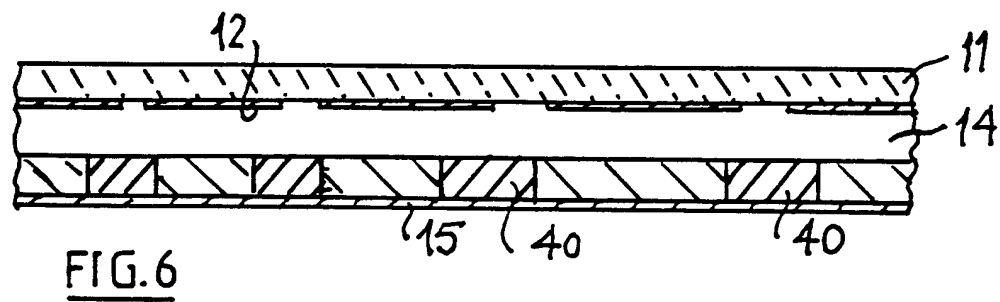
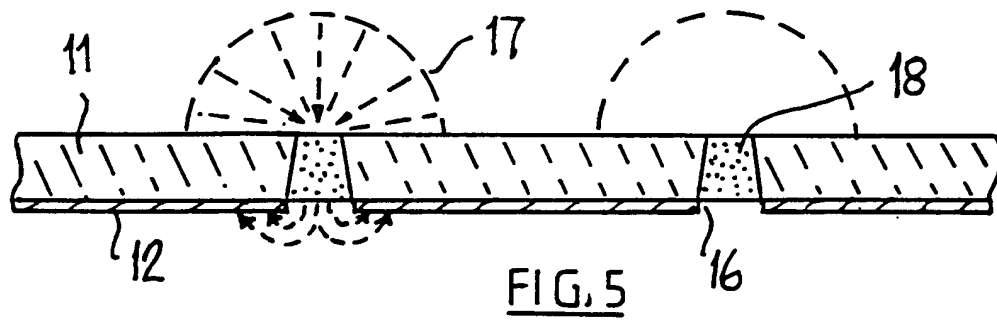
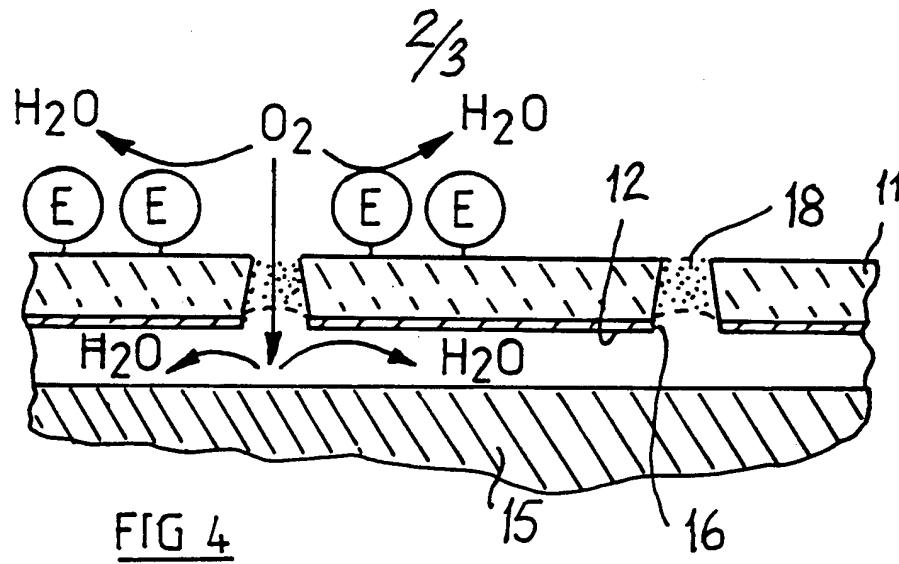
of claim 1 incorporating at least one enzyme for the determination of a biological species.

11. A composite electrode comprising a gas-permeable membrane fabricated in accordance with the method of claim 5 1 and means for providing a supported liquid-liquid interface adjacent thereto.

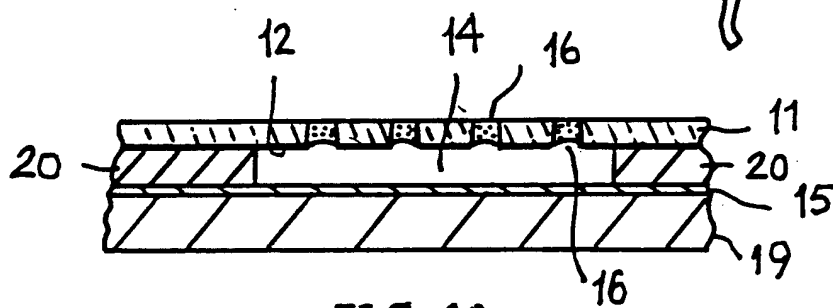
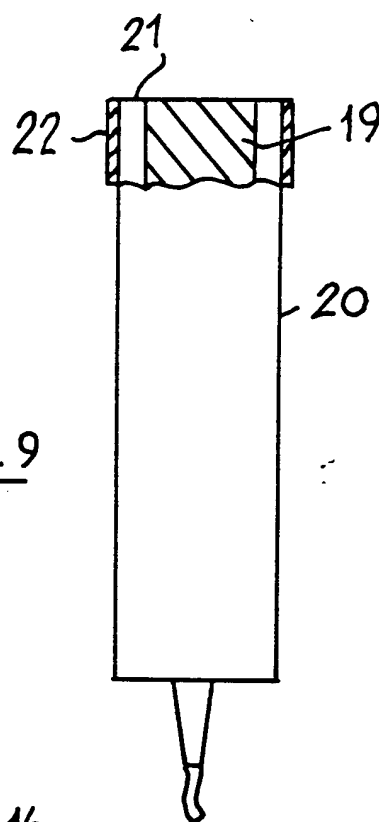
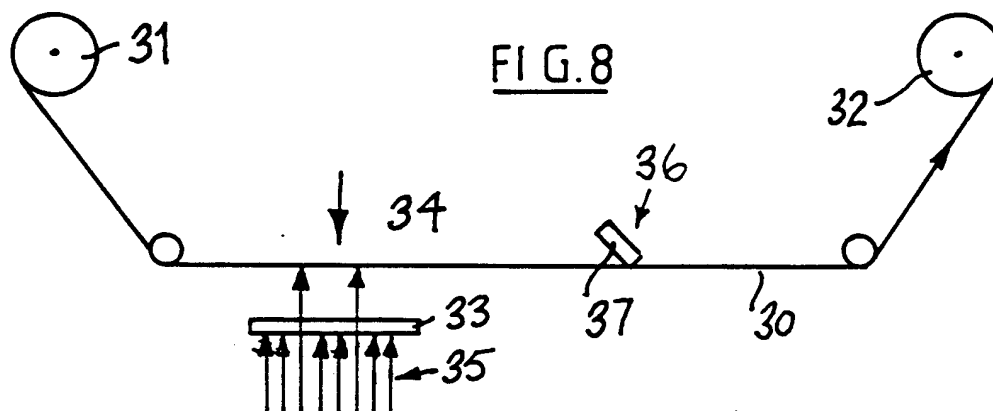
12. A disposable oxygen sensor incorporating a gas-permeable electrode according to claim 8 and incorporating a defined cavity which may contain microbes.

10 13. An amperometric gas electrode according to claim 8 in which the metallised layer serves as both gas barrier and electrode.





3/3



INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 93/02076

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 G01N27/49 C12M1/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 494 382 (PPG INDUSTRIES, INC.) 15 July 1992 cited in the application	1-4,6-10
A	see the whole document ---	11,13
Y	WO,A,91 08474 (ECOSSENSORS LIMITED) 13 June 1991 cited in the application	1-4,6-10
A	see the whole document ---	11,13
Y	EP,A,0 237 914 (BAYER DIAGNOSTIC + ELECTRONIC) 23 September 1987	1-4,6-10
A	see the whole document ---	11,13
A	EP,A,0 154 982 (HONEYWELL INC.) 18 September 1985 see the whole document ---	1-4, 6-11,13
	--- -/--	

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

2 February 1994

Date of mailing of the international search report

18.02.94

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Bosma, R

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 93/02076

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE,A,32 47 722 (ME MEERESTECHNIK-ELEKTRONIK GMBH) 28 June 1984 see the whole document -----	1-4, 6-11,13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 93/02076

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0494382	15-07-92	US-A- 5246576 JP-A- 4353755	21-09-93 08-12-92
WO-A-9108474	13-06-91	AU-A- 6880091 EP-A- 0504196	26-06-91 23-09-92
EP-A-0237914	23-09-87	DE-A- 3609402 JP-A- 62231157	24-09-87 09-10-87
EP-A-0154982	18-09-85	US-A- 4521290 CA-A- 1223038 JP-A- 60211352	04-06-85 16-06-87 23-10-85
DE-A-3247722	28-06-84	NONE	